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Evaluation of thyroxine (T_4) and progesterone-17-α-OH circadian rhythm in swine females (*Sus scrofa domestica* – Linnaeus, 1758)*

Avaliação do ritmo circadiano de tiroxina (T_4) e 17-α-OH progesterona em fêmeas suínas (*Sus scrofa domestica* – Linnaeus, 1758)

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SUMMARY

Aiming at the evaluation of the occurrence of thyroxine and progesterone-17-α-OH circadian rhythm, 4 adult crossbred swine females (non-pregnant and showing no estrous signs) were studied, reared and kept under a industrial management system. Hormone determinations were assessed by solid phase radioimmunoassay (RIA)[a]. Thyroxine serum analysis showed the highest concentrations around 3:00 PM and decreased until reaching the lowest values at the 0:00 to 4:00 AM interval. Regarding progesterone-17-α-OH, the highest concentrations were observed at 3:00 AM, showing a progressive decrease along the day until reaching lower values at the 12:00 to 3:00 PM interval.

UNITERMS: Circadian rhythm; Hormone; Swine.

INTRODUCTION

The reproduction related hormones in mammals show a great variation (pertaining their levels) even when the physiological status is taken into account during a 24-hour period[2]. Turek; Cauter[12] reported that the development of sensitive and precise hormone assays are making it possible to obtain a more accurate analysis of hormone profiles from a sequential collection of samples (from the same animal) at certain time intervals. Such an approach reveals that plasma levels from most known hormones display frequency and amplitude episodic fluctuations, referred to in the literature as pulses or peaks. The biological phenomenon, which can be rhythmically expressed within a 24-hour period, under natural and/or artificial conditions, can be defined as circadian rhythm[3]. According to Turek; Cauter[12] most hormone rhythms studied under constant environmental conditions have demonstrated the occurrence of circadian rhythms, indicating that a certain temporal system must be involved in its generation. Thus, under normal environmental conditions the circadian rhythm length is equal to daylength but some factors from the environment must be involved to synchronize the biological rhythmicity. For most mammals, the bright-dark cycle appears to be the most important factor for the synchronization of the biological rhythm, although other external agents, such as food and temperature, have been identified.

The suprachiasmatic nucleus (SCN), a bilateral structure positioned at the anterior hypothalamus, contains the biggest circadian "regulator" controlling most of the endogenously generated rhythms. This structure receives direct neural signaling from the retina via retino-hypothalamic tract (RHT) which is involved in the transmission of bright-dark information to the SCN.

Krieger; Hauser[8] showed that biological rhythms appeared to consist of several secretory waves varying in frequency and amplitude throughout the day. Circadian variations have been well studied, mainly those from adrenocortical function which result in ACTH secretion[4] and demonstrated for several mammalian species, including murine[14], equine[7,1], cattle[6] and human being[10], among others. Vicente *et al.*[13] studied the occurrence of progesterone, androsterone and cortisol circadian rhythmicity from blood samples collected at 4-hour intervals, during one day, from 4 crossbred swine females (reared under industrial conditions). It was observed that those 3 hormones displayed a similar behaviour, achieving higher concentrations at the 00:00 to 03:00 AM interval, gradually declining during the day until reaching the lowest values at the 12:00 to 03:00 PM interval.

Studies pertinent to diurnal rhythms of thyroid hormones in dogs were elsewere described[9,5]. The former reported that T_4 diurnal peak concentrations occurred at noon. In contrast to the latter, which observed that it seemed unlikely that the diurnal cycle could exist from 08:00 AM to 08:00 PM. Data related to circadian

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[a] Coat-a-Count® kit – Diagnostic Products Co., Los Angeles, CA, USA.

rhythm during the estrous cycle in gilts and sows, involving progesterone-17-α-OH and thyroxine, were unavailable. At that opportunity we attempted to assess the biological circadian rhythmicity occurrence.

The major goal of the present study was to establish useful parameters for better knowledge and exploitation of this species and contribute to the progress of Comparative Physiology.

MATERIAL AND METHOD

Animals and Management

Experimental animals consisted of 4 adult non-pregnant Landrace x Large White crossbred females, showing no signs of standing heat. The gilts* randomly assigned to the hormonal circadian rhythm evaluations, were kept under strict feedlot in individual pens for 2 weeks. At the end of this period each animal was submitted to 6 venal punctions at a 4-hour interval, performed at 00:00, 04:00 and 08:00 AM and 12:00, 04:00 and 08:00 PM.

Blood Sampling

Blood sample collection was carried out by punction of cranial vena cava, through disposable 20 ml syringes (100 x 10 mm needles). Approximately, a volume of 20 ml/sample was taken and immediately transferred to sterile glass tubes. Samples were carefully carried to the laboratory, where the serum was centrifuged at 1,600x g for 5 minutes. Immediately after, the supernatant was transferred into 4 sterile glass flasks of similar volume and stored at -18ºC until assayed.

Hormone Analysis

Hormone assays were performed by means of radioimmunoassay (RIA) technique, employing a set of commercial reagents[a], developed for quantitative evaluation of progesterone-17-α-OH and T_4, without any kind of chemical extraction or purification process, using I^{125} as a radioactive tracer.

To perform the hormone analysis, samples were thawed (together with specific reagents for each hormone) until reaching room temperature. Serum flasks were gently shaken before use. Procedures for the assays were conducted as described by the respective protocol. Results were obtained by a specific computer program.

Performance evaluations of the assays were based upon: (1) Maximum binding percent (% BO); (2) Non-specific binding percent (% LNE); (3) Intra-assay coefficient of variation for low (% CV1) and high (% CV2) values; and (4) Sensitivity level of the test, estimated from the counting of two pairs of tubes mean and SE (from the maximum binding calibrator, zero calibrator). The sensitivity being defined as the hormone's lowest apparent concentration at two SE from the mean.

Statistical Analysis

The Cosinor method[11] was employed to assess the occurrence of the circadian rhythm.

RESULTS

Progesterone and thyroxine concentrations showed distinct patterns. Regarding to progesterone-17-α-OH it was observed higher concentrations around 03:00 AM, gradually decreasing during the day until reaching the lowest values at the 12:00-03:00 PM interval and increasing again at night. In contrast, thyroxine concentrations achieved the highest serum levels around 03:00 PM, then decreased until reaching the lowest values at the 00:00-04:00 AM interval, as shown in Fig. 1 and 2 (progesterone-17-α-OH and thyroxine, respectively).

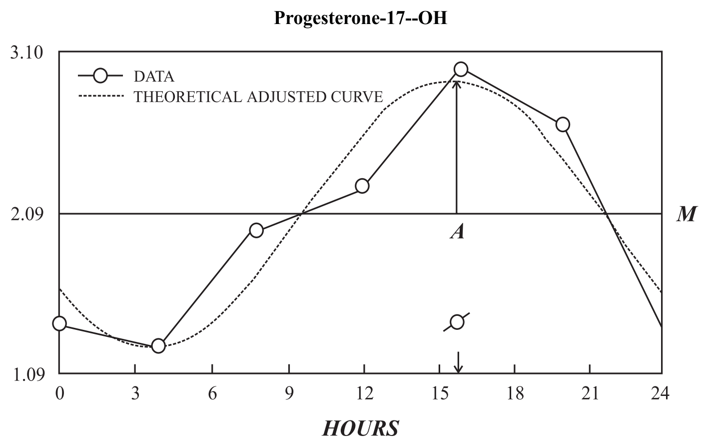

Figure 1
Biological rhythmicity as an adjusted co-sine function and progesterone-17-α-OH values, during a 24-hour period from blood serum of 4 swine females. M = mesor (adjusted curve mean value); A = amplitude (distance between maximum or minimum value and mesor); Ø = acrophase (time of occurrence of the adjusted curve maximum value).

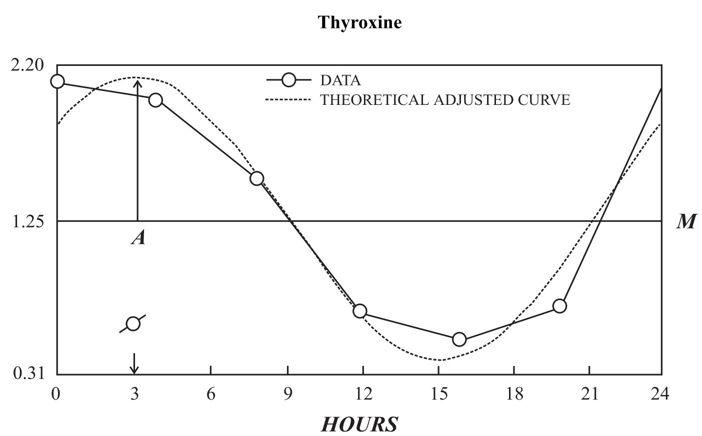

Figure 2
Biological rhythmicity as an adjusted co-sine function and thyroxine values, during a 24-hour period from blood serum of 4 swine females. M = mesor (adjusted curve mean value); A = amplitude (distance between maximum or minimum value and mesor); Ø = acrophase (time of occurrence of the adjusted curve maximum value).

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* Humus Agrícola Enterprises, Pitangueiras/SP.
[a] Coat-a-Count® kit - Diagnostic Products Co., Los Angeles, CA, USA.

DISCUSSION

Hormones related to reproduction show a great variation, pertaining their levels, even when considering a certain physiological status during a 24-hour period, according to observations from Cauter; Aschoff[2]. Thus, the circadian rhythm variations, mainly those from adrenocortical function, which result in ACTH secretion[4], have been well studied in several mammalian species such as murine, equine, bovine, swine and human being[5,6,7,9,10,13,14]. Other authors, such as Krieger; Hauser[8], demonstrated that the biological rhythm seems to consist of several secretory waves which vary in amplitude and frequency during the day. Results from the present work displayed, for progesterone-17-α-OH, higher serum levels around 03:00 AM, decreasing gradually throughout the day until achieving the lowest values at the 12:00-03:00 PM interval, then increasing at night. Serum levels pertaining to T_4 were higher around 03:00 PM and lower at the 00:00-04:00 AM interval, as shown in Fig. 1 and 2, respectively. These results are not quoted in the reviewed literature. Nevertheless, Nachreiner[9] reported that for dogs a diurnal peak in T_4 concentrations occurs at noon but those results are controversial because other authors[5] observed that it is unlikely to exist diurnal cycles (from 08:00 AM to 08:00 PM) for thyreoid hormones. On the other hand, Vicente *et al.*[13] studied the occurrence of rhythmicity for progesterone and other hormones and observed higher values from 00:00 to 03:00 AM, gradually decreasing during the day until reaching the lowest values at the 12:00-03:00 PM interval. This evaluation allowed us to deduce that the results observed for progesterone are similar to those shown in Fig. 1, what in our understanding makes sense, because this hormone is a progesterone byproduct and in this way it was expected that the results related to the circadian rhythm didn't differ.

Finally, it is relevant to explain that the circadian rhythm pattern observed for progesterone-17-α-OH and thyroxine are different, which make us wonder that the ovarian secretion pattern doesn't follow the same one as for the thyroid, although no references were found on this subject. Nevertheless, we believe that the thyroidean hormones can affect, even in an indirect way, the ovarian steroid production.

CONCLUSION

The results observed in the study of biological rhythmicity, within the conditions of the present work, allowed us to admit the following hypothesis:

- The methodology and the kit of reagents applied for the hormone assays were effective;
- The circadian rhythm hormone profiles (progesterone-17-α-OH and thyroxine) were contrasting. The findings suggest that the thyreotrophic hormones display a rhythmic pattern different from the gonadotrophic ones.

RESUMO

No presente trabalho foram utilizadas quatro fêmeas suínas, adultas, mestiças, não-gestantes e sem sinais clínicos de estro, criadas e mantidas sob condições industriais de criação. Objetivou-se avaliar a ocorrência de ritmicidade biológica circadiana para tiroxina e 17-α-OH progesterona. Os ensaios para dosagens hormonais foram executados utilizando-se a técnica de radioimunoensaio (RIE) em fase sólida e para isso foi empregado conjunto de reagentes comerciais (Coat-a-Count®). As análises séricas de tiroxina mostraram valores mais elevados ao redor das 15 horas, decrescendo a partir dai até atingir níveis menores no intervalo da zero às 4 horas. Quanto a 17-α-OH progesterona, observaram-se níveis mais elevados por volta das 3 horas, decrescendo gradativamente ao longo do dia, até atingir menor concentração no intervalo das 12 às 15 horas.

UNITERMOS: Ritmo circardiano; Hormônios; Suínos.

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